Journal of Research on the Lepidoptera 3 (3) :159-172, 1964

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ADULT OVIPOSITION RESPONSES IN *PIERIS RAPAE*

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Differential responses of the larvae of *Pieris rapae* to various food plants, and to various mustard oils have been shown previously (Hovanitz and Chang, 1962, 1963, 1964). The responses of the adult females with regards oviposition have been somewhat a different problem, in as much as there is involved not only an odiferous attractant, but also, the physical condition of the substrate. In this paper, several experiments are described, each of which tests females of *Pieris rapae* for a different environmental factor related to oviposition. All of these experiments were carried out by the junior author.

TEXTURE OF MATERIALS

The response of females to different textured materials was tested by covering small potted mustard plants (as the odiferous attractant) with various substances. These included white filter paper, wax paper, marquisette, plastic film with minute holes and an agar coating. As a control, one plant was left uncovered. These plants were placed in a cage, 46cm X 46cm X 20cm, along with five female *P. rapae*. After five days, the number of eggs laid on each plant, covered or not, was counted. The females were mated before the experiments were begun and fed diluted honey daily.

The results (Table 1) indicate that the white filter paper was a poor attractant. If there were no difference in attraction of the six plants, the total eggs (1900) would be equally divided into 316.67 eggs per plant, deviating only by chance. The deviation for the white filter paper is —288.67 eggs, a very significant quantity. The significance of the deviation on the remainder has been tested by χ^2, using the hypothesis that the

[1]Aided by a grant from the National Science Foundation, Washington, D. C.

	Texture	N
1.	White filter paper	28
2.	Wax paper	300
3.	Agar coat	342
4.	Plastic sheet with holes	363
5.	Marquisette	417
6.	Uncovered	450

Table 1. Oviposition responses of Pieris rapae to the different materials covering mustard plants. Number of eggs.

	Position						
	1	2	3	4	5	6	Total
No. eggs	404	543	561	515	553	526	3102
Percent	13.0	17.5	18.1	16.6	17.8	17.0	100

Table 2. Oviposition responses to the direction of the sun and the wind. (Fig. 1) Eggs laid by 20 females in 10 days.

five plants had an equal chance for being the recipient in egg laying. The deviation of each plant from the expected number of eggs (374.4) was calculated. The χ^2 value of 435 for 4 degrees of freedom gives a probability greatly less than 1 chance per 10,000 that such deviation might have occurred due to chance alone. Testing for a single plant (covered with wax paper) having a deviation of 74.4, indicates a χ^2 of 18.47, $p = < .0001$ that even this single deviation would have occurred due to chance alone. A plant having a deviation of only 42.6 (covered with marquisette indicates a χ^2 value of 6.06, $p = .015$ that this deviation could have occurred due to chance alone. It is clear that all the plant coverings create a significant decrease in the number of eggs laid as compared with no covering at all, but that based upon chance alone, agar covering, plastic covering and marquisette are not highly significantly different, one from the other. Nevertheless, on the basis of the figures, the coverings least offensive to the female Pieris are (in order):

marquisette (probably because of the large holes for dissemination of odors and moisture.)
plastic film with holes (probably because of the small holes for dissemination of odors and moisture).
agar coat (probably because of its permeability).
wax paper (probably because of its permeability).
Most offensive is white filter paper.

ARTIFICIAL SUBSTRATES

In order to test the effects of various odiferous substances, an artificial substrate has been designed which will be attractive to the females, will permit the odors to be effective and will not be wet so as to gum up the legs and wings of the adult. This was done by matching the surface of a leaf as much as possible by providing a smooth surface (plastic film), partially permeable (full of minute holes) and with a moist gelatinous material underneath (agar gel). The cover reduces the rate of evaporation, prevents the wings and legs of the insect from becoming trapped and permits a smooth hard surface for egg attachment.

The substrate was made out of a shallow plastic dish 10cm. in diameter with 30cc water holding capacity. A medium consisting of 1.5% agar and water, together with varying amounts of attractant and colors was provided.

Each such dish was mounted on a cork placed on top of a 120cc flask and six such flasks were arranged circumferentially in a marquisette cage 46cm X 46cm X 20cm in size (Fig. 1, 2).

In each of the experiments, the medium was changed and the number of eggs was counted daily (Fig. 4).

The adults used in all the experiments described in this paper were from a kale-strain described in previous papers (Hovanitz and Chang, 1963).

The data indicated in all subsequent tables are based on 10 days cumulative results, as the number of eggs laid each day by the females varied due to changes in weather conditions and age of the females.

EFFECT OF DIRECTION OF SUN AND AIR MOVEMENT

The purpose of this test was rather coincidental to later ones, namely to test whether or not the plants in the various geographical positions in the cage would be affected by the southerly position of the sun as it moved from east to west during the day; also, to test any possible effect of a unidirectional air movement in the greenhouse.

Fig. 1. Oviposition cage from above showing six different colored media covered with plastic film.

Fig. 2. Oviposition cage from side showing media plaques and butterflies.

Fig. 3. Eggs and newly hatching larvae on plastic film from top of artificial medium plaque. Note the many eggs around edge. These are normally laid under the edge but are here shown turned up as the plastic film is straightened out.

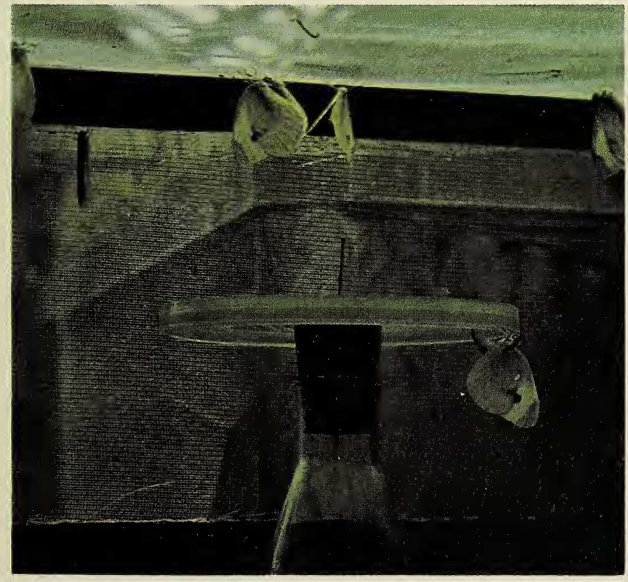

Fig. 4. Close-up of single artificial medium unit showing female laying egg.

Six substrate dishes were arranged in the cage circumferentially, and numbered in order starting from number 1 at the north, and number 4 at the south (Fig. 5). Thus, numbers 2 and 3 were at east side and numbers 5 and 6 at the west side. The air movement was in the direction from south to north.

The test dishes were all loaded with an identical artificial substrate for egg laying, which was very successful. Each medium contained a 2% water soluble fraction of ground mustard seeds (*Brassica nigra*), 0.33 percent of green food-coloring fluid and 1.5 percent agar solution.

The water soluble fraction of ground mustard seeds was prepared in the following way:

For six media dishes, six grams of finely ground mustard seeds are used. This is shaken continuously for five to ten minutes and then filtered. Each dish contains 6cc of such extract, mixed with 24cc of green-colored agar solution. After cooling and solidification, the dish is covered with a plastic film which has previously been poked ful of tiny holes by insect pins.

Three thousand, one hundred and two eggs were laid by twenty females in this experiment. These were distributed in the six positions as shown in Table 2. Position number 1 had the fewest eggs at 404, while position number 4 was second lowest at 515. With the assumption that all positions had an equal chance, the data were tested by chi-square, giving the result: $\chi^2 = 32.4$ (5 degrees of freedom), $p = < .0001$, a very significant deviation from chance.

Testing the assumption that perhaps only number 1 deviates significantly from the remainder, a test was made on the remaining five. These gave a ^{2} value of 7.6 for 4 degrees of freedom, $p = .07$, which is not highly significant. This would indicate that the basic deviation is in number 1, away from the sun and the direction of air movement. However, since number 4 is second lowest, number 1 and number 4 were combined and compared with a combination of 2, 3, 5, 6. The four just mentioned are at "sides" as compared with 1 and 4 which are at the "front" and "back" as compared with the direction of sun and air movement. Combined in this way, number 1 and number 4 were significantly different from the others, $\chi^2 = 19.2$ (2 degrees of freedom), $p = < .0001$.

In all subsequent tests, therefore, to eliminate the effects of this unidirectional environmental factor, the cage was rotated ninety degrees daily. The cage was therefore rotated completely

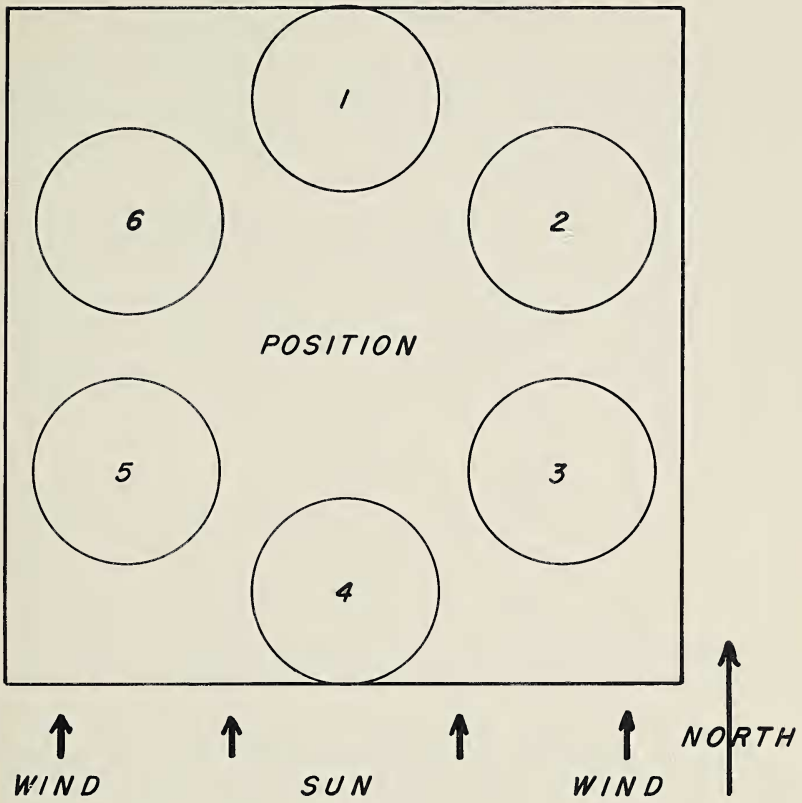

Fig. 5. Diagram illustrating the relative positions of artificial oviposition mediums in relation to direction of wind movement and sun direction in test area.

2½ times during the course of the experiment. In any event, the maximum difference between any of the positions was 5 percent of the total eggs laid.

RESPONSE TO COLOR

To test the response of the female *Pieris rapae* to color of the substrate, media were prepared as above, 2 percent water soluble fraction of mustard seeds, 0.33 percent color fluid and 1.5 percent agar solution.

Red, yellow, blue and green colors were obtained directly from Shilling food colors (McCormick and Co., Inc.). Yellow-green color was obtained by mixing the two in a 1 : 1 ratio. Different shades of blue and green were obtained by blending these two in different proportions, for example, G_1 B_4 was one part green to four parts blue. No color was added to "white" color medium (Fig. 1).

Experiment Number 1: The colors red, yellow, white, blue, and yellow-green were tested, using 15 females for egg laying. Of the total of 734 eggs obtained (Table 3), 630 or 85.83 percent were on yellow-green, 67 or 9.12 percent were on blue and 35 or 4.77 percent were on white. Yellow and red were negligible with only one each. Since yellow alone was insignificant, the high selection on yellow-green is presumed to be selection for green.

Experiment Number 2: The colors green, yellow-green, blue, white, yellow and red were tested. Of 1114 eggs, 478 or 42.9 percent were on green, 356 or 30.2 percent were on yellow-green, 260 or 23.3 percent were on blue, 40 or 3.6 percent were on white and none at all were on yellow or red. It appears as if green is most favored and that blue is second. Yellow is not only not favored but may be repellent, as shown by the addition of yellow to green, decreasing the effectiveness of the green. Here, however, since the green is combined 1 : 1 with yellow, the decrease may be due to 50% reduction in the quantity of green pigment.

Experiment Number 3: Green, blue and various combinations of these two were tested in this experiment. Green was six times more effective than blue (18 percent for green over 3 percent for blue) in a total of 620. A series of combinations of blue and green were made up ranging from one part green: 4 parts blue to 4 parts green: 1 part blue. The addition of blue to green had a slight (or no significant) effect on raising the selective response (18 percent to 19 percent). The proportion of 2 parts green to

A.

	Yellow green	Red	Yellow	White	Blue	Total
No. eggs	630	1	1	35	67	734
Percent	85.83	0.14	0.14	4.77	9.12	100

15 females

B.

	Green	Yellow green	Blue	White	Yellow	Red	Total
No. eggs	478	336	260	40	0	0	1114
Percent	42.9	30.2	23.3	3.6	0	0	100

15 females

C.	Blue	G_1B_4	G_2B_3	G_3B_2	G_4B_1	Green	Total
No. eggs	21	86	165	117	120	111	620
Percent	3.4	13.9	26.6	18.9	19.4	17.9	

6 females

Table 3. Oviposition responses of Pieris rapae to different colors.

three parts blue was significantly better in illiciting a response, however, to 26.6 percent of the total eggs. Any greater blue (or decrease in green) however, brought about a drop in selection (14 percent for 1 part green to 4 parts blue). The fact that blue plus green has a greater effect than either alone is a point of interest.

RESPONSES TO CONCENTRATION OF EXTRACT

Two tests were made on varying amounts of mustard-seed extract in the media. For example, 2 percent extract media contained 6cc of 10 percent mustard extract and 24cc yellow-green colored agar solution; the 4 percent extract media contained 12cc of 10 percent extract and 18cc agar solution. In addition to these, a test was made by adding 0.1 gm. of finely ground mustard seeds directly to the agar solution, and controls of 6cc distilled water were used.

In the first series of tests, test plates consisted of the extract concentrations ranging in percentage from 0.1, 0.5, 1 and 2, ground mustard-seed and distilled water. The 2 percent concen-

tration was significantly higher than all the others with response at 31 percent of all eggs (70/225); the lowest was the "ground mustard-seeds", 8 percent (17/225) (Table 4).

			percent concentration				
A. [5 ♀♀]	0.1	0.5	1.0	2.0	0.1 gm. grnd. must. seeds	H_2O	Total
eggs	26	30	43	70	17	39	225
percent	11.5	13.3	19.1	31.1	7.6	17.3	
B. [16 ♀♀]	1.0	2.0	4.0	6.0	H_2O	Total	
eggs	208	459	408	369	213	1657	
percent	12.6	27.7	24.6	22.3	12.9		

Table 4. Oviposition responses of Pieris rapae females to different concentrations of water soluble fractions of ground black mustard seeds.

	Test A		Test B	
	Number of eggs	Percent	Number of eggs	Percent
Water soluble fraction of mustard seeds	553	72.19	1564	74.3
Distilled water	213	27.81	541	25.7
Total	766	100	2105	100
No. females	10		20	

Table 5: Oviposition responses of Pieris rapae to the artificial substrate with and without attractant.

In the second series of tests, the concentration of extract was increased to percentages of 1, 2, 4 and 6, again with distilled water as a control. The 2 percent concentration was again highest in eggs deposited although 4 and 6 percent were not far behind. One percent and distilled water were lowest (Table 4).

Two tests were made to detect the ability of the females to distinguish between plates with and without mustard-seed extract. The fact that plates in the previous experiments had eggs laid on them even though no extract was present leads one to believe that proximity of extract-containing plates induces oviposition activity, even on plates not actually permeating mustard odors. Three plates of 2 percent extract with 1.5 percent green colored agar were alternated around the cage with plates using only the colored agar.

In the first test, of 766 eggs laid, 553 or 72 percent of the eggs were laid on the mustard fraction plates (Table 5).

In the second test of 2105 eggs laid, 1564 or 74 percent were laid on the mustard extract plates, approximately the same proportion as in the first test (Table 5).

The interesting observation here is that about one-fourth of all the eggs were laid on the plates without any attractant. This would indicate that the moisture, and attractive qualities of the plastic film alone, were sufficient, although one can not rule out the proximity of adjacent plates containing mustard extract as having some effect here.

COMPARISON OF ARTIFICIAL MEDIA AND NATURAL LEAVES

There is no very good way of comparing directly the comparative attractive qualities of the artificial media plates and leaves of the Pieris host plant. Nevertheless, an indirect method is possible by placing in one cage a full set of six plates with attractant and color at optimum concentrations. In another cage of identical size and conditions are placed six small black mustard plants. In each of these two cages are placed 15 adult females for egg laying over a comparable period of time where the two cages are set side by side.

The main difference in physical aspects of the two set-ups is the multi-level aspect of the leaves on the plants as compared with the single-level aspect of the plates (Fig. 3).

Two experiments were arranged. In the first (Table 6), 3974 eggs were laid of which 1829 (46.02 percent) were laid on

artificial media. In the second, 5615 eggs were laid of which 2689 (47.89 percent) were laid on artificial media.

These data show that the artificial medium is nearly as successful as the plants themselves in inducing oviposition.

Table 6A	Exp.	No. females	Total no. eggs	Eggs/female
Artificial media				
	1	15	1829	121.9
	2	15	2689	179.3
Total		30	4518	150.6
Black mustard leaves	1	15	2145	143.0
	2	15	2926	195.1
Total		30	5071	169.0

Table 6 B	artificial media		leaves		Total
Experiment 1	1829	46.02	2145	53.98	3974
Experiment 2	2689	47.89	2926	52.11	5615

Table 6 : Oviposition responses of Pieris rapae to the artificial media and the natural food plant.

	-5 10	-6 10	-7 10	-8 10	H 0 2	2 percent mustard fraction	Total
No. eggs	241	125	119	144	215	593	1437
Percent	16.8	8.7	8.3	10.0	15.0	41.3	

25 Females

Table 7: Oviposition responses of Pieris rapae to various concentrations of allyl-iso thiocyanate.

RESPONSE TO ALLYL-ISOTHIOCYANATE

The media as previously compounded was made up, substituting various dilutions of pure natural mustard oil (allyl-isothiocyanate) produced by Fritsche Brothers, Inc., New York. All media contained 1.5 percent yellow green agar solution. Dilutions of the mustard oil were 10^{-10}, 10^{-6},10^{-7} and 10^{-8}. In the same experiment were placed plates containing 2 percent mustard seed fraction and water alone.

Of a total of 1437 eggs, 593 or 41.3 percent were laid on the one plate having mustard seed fraction. The next highest was the 10^{-5} dilution (241, or 16.8 percent) which was not much different from water (215 or 15.0 percent). The other three concentrations of mustard oils were considerable less effective than water, inducing ovipositions of 119, 125 and 144 eggs respectively. It would appear that these concentrations of mustard oil are actually repellent to the female *P. rapae* despite the inducements to the larvae.

SUMMARY

1. Differential responses of adult female *Pieris rapae* are shown to various substrata, and other environmental factors.

2. Mustard plants were covered with marquisette, plastic film with holes, agar coating, wax paper and white filter paper. Egg laying was greatest on the coverings in the order shown.

3. A method of producing a desirable artificial substratum is described.

4. The effect of the sun and air movement was shown to be significant.

The effect of different colored substrata was tested. Green is preferred greatly with blue second in preference. Yellow may be repellent and red is of no significance. A combination of green-blue was found to be more favored than green alone but this may be due to a lowered concentration of green.

6. Optimum concentration of mustard seed soluble extract was tested. A 2 percent concentration was found most favorable.

7. In a comparison of artificial media with natural leaves, natural leaves were found only slightly superior to the artificial as judged by the number of eggs laid. This may be due to surface area exposed, however.

8. Various concentrations of allyl isothiocyanate were tested. None elicited better response significantly higher than water alone, and all were lower than mustard seed soluble extract. Three concentrations indicated that the mustard oil may actually be repellent at those concentrations.

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